



⑯ Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Publication number:

O 238 868
A2

⑫

EUROPEAN PATENT APPLICATION

㉑ Application number: 87102543.3

㉓ Int. Cl.4: C 07 C 119/02, C 07 C 103/38,
A 61 K 31/275, A 61 K 31/16,
A 01 N 37/18, A 01 N 47/40

㉒ Date of filing: 23.02.87

BEST AVAILABLE COPY

㉔ Priority: 25.02.86 JP 39382/86
08.04.86 JP 81522/86

㉕ Applicant: Zaidanhōjin Biselbutsu Kagaku Kenkyukai,
14-23 Kamiosaki 3-chome, Shinagawa-ku Tokyo (JP)

㉖ Date of publication of application: 30.09.87
Bulletin 87/40

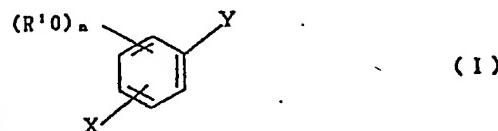
㉗ Inventor: Umezawa, Hamao, 23, Toyotamakita 4-chome
Nerima-ku, Tokyo (JP)
Inventor: Tatsuta, Kunjaki, 26-7, Matsunoki 2-chome
Suginami-ku, Tokyo (JP)
Inventor: Takeuchi, Tomio, 5-1-11, Higashitoganda
Shinagawa-ku, Tokyo (JP)
Inventor: Isehiki, Kunio, 2-13-1332,
Fujisawa-selbudanchi Shonan Life Town, 3910, Oba
Fujisawa-shi (JP)
Inventor: Imoto, Masaya, Green Heights 102 5-27-11,
Kungahara, Ota-ku Tokyo (JP)

㉘ Designated Contracting States: DE FR GB

㉙ Representative: Patentanwälte Müller-Boré, Deufel,
Schön, Hertel, Lewald, Otto, Isartorplatz 6,
D-8000 München 2 (DE)

㉚ New physiologically active substance erbstatin analogous compounds.

㉛ The present invention provides new physiological active substance erbstatin analogue compounds of a general formula (I), which have excellent tyrosine-specific protein-kinase inhibitory activity, antitumor activity and antimicrobial activity.



In which R¹ represents a hydrogen atom, a lower alkanoyl group or a lower alkyl group; n represents a positive integer of 1 to 3;
X represents a hydrogen atom or a halogen atom;
Y represents a group of formula -CH=CH-NC, -CH=CH-NHR² or -CH₂-CH₂-NHR²
(where R² represents a formyl group or a lower alkanoyl group; with the exception of the cases where X is hydrogen atom, (R¹O)_n group is 2,5-dihydroxy group and Y is -CH=CH-NHCHO or -CH₂-CH₂-NHCHO).

The present invention further provides a tyrosine-specific protein-kinase inhibitor, tumoricide or bactericide containing at least one compound of the said formula (I).

EP O 238 868 A2

0238868

NEW PHYSIOLOGICAL ACTIVE SUBSTANCE
ERBSTATIN ANALOGUE COMPOUNDS

FIELD OF THE INVENTION

The present invention relates to new physiological active substances, more concretely, to those having tyrosine-specific protein-kinase inhibitor activity, antitumor activity and antimicrobial activity.

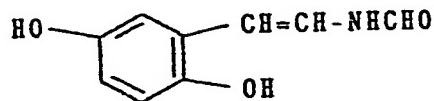
BACKGROUND OF THE INVENTION

Regarding antitumor substances, a lot of compounds have heretofore been put to practical use as medicines. However, these are not always satisfactory in view of the pharmaceutical effect and/or the harmful aftereffect thereof. Accordingly, diversified studies have been made in order to develop more excellent antitumor substances.

For instance, Bishop, J.H. reported that a certain kind of genetic products have a tyrosine-specific protein-kinase property (A. Rev. Biochem., 52, 301-354 (1983)). In addition, Cohen, S. et al. reported that a tyrosine-specific protein-kinase participates in the process of the propagation of cells by a variety of cell growth factors as a signal substance (J. Biol. Chem., 257, 1523-1531 (1982)). On the other hand, the present inventors screened, paying their attention to the said phenomena, inhibitors against tyrosine-specific protein-

0238868

kinase activity widely from the natural world and found that a substance of the formula:

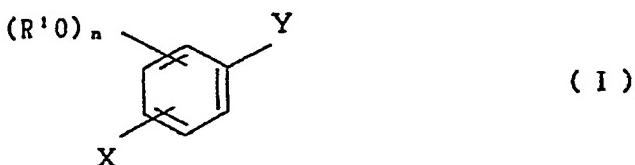


as produced by microorganisms belonging to the genus Streptomyces can inhibit the said protein-kinase activity in a low concentration and further has antimicrobial activity and antitumor activity. This was designated MH435-A and was already proposed (Refer to Japanese Patent Application No. 147039/85).

In addition, the present inventors formed a variety of MH435-A substance analogues by chemical synthesis in order to obtain compounds having a higher physiological activity and have found that some benzene compounds which are substituted by an isonitrilevinyl group, formamidovinyl group, formamidethylene group, lower alkanoylaminovinyl group or lower alkanoylaminethylene group on the benzene ring and which are further nuclear-substituted by optionally protected hydroxyl group(s) or a halogen atom are in no way inferior to the above-mentioned MH435-A substance in the physiological activity and thus have achieved the present invention.

SUMMARY OF THE INVENTION

The present invention provides compounds of a general formula (I), which have an inhibitory activity against tyrosine-specific protein-kinase (hereinafter referred to as "TPK") and further have antimicrobial and/or antitumor activities:



(in which R¹ represents a hydrogen atom, a lower alkanoyl group or a lower alkyl group; n represents a positive integer of 1 to 3;

X represents a hydrogen atom or a halogen atom;

Y represents a formula -CH=CH-NC, -CH=CH-NHR² or -CH₂-CH₂NHR² (where R² represents a formyl group or a lower alkanoyl group);

with the exception of the cases where X is hydrogen atom, (R¹O)_n group is 2,5-dihydroxy group and Y is -CH=CH-NHCHO or -CH₂-CH₂NHCHO).

Accordingly, the present invention provides benzene compounds which are substituted by an isonitrilevinyl group, formamidovinyl group, formaidoethylene group, lower alkanoylaminovinyl group or lower alkanoylaminooethylene group on the benzene ring and which are further nuclear-substituted by optionally protected hydroxyl group(s) and/or a halogen atom.

0238868

DETAILED DESCRIPTION OF THE INVENTION

Among the above-mentioned compounds of the present invention, those having an isonitrilevinyl group not only have an especially strong antimicrobial activity but also are important as intermediates in synthesis of the compounds having a formamidovinyl group or formamidoethylene group. In the other compounds, those having a formamidovinyl group or lower alkanoylamidovinyl group are characterized by the strong inhibitory activity against TPK(hereinafter referred to as "TPK-I") and the strong antitumor activity.

In the present invention, the lower alkanoyl group and the lower alkyl group mean that the alkyl group part is an optionally branched alkyl group having 1 to 5 carbon atoms. Typical examples of these groups are acetyl, propionyl, n-butyryl, iso-butyryl, t-butyryl, pentanoyl, methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl and pentyl groups.

Among these groups, acetyl group and methyl group are especially preferred as the protective group of the hydroxyl group. However, the compounds having free hydroxyl group(s) are desired in view of the activity in vitro.

The number of the substituents of the said hydroxyl groups may be 1 to 3. In particular, the compounds having two hydroxyl substituents, or that is, di-hydroxyl-substituted compounds are preferred.

0238868

The N-acyl group is significant in the point of the elevation of the stability of the compounds of the present invention, and the selection of the said group is not limitative since the preferred one is to be determined, depending upon the kind of the basic structure of the compounds. However, the hydroxyl group(s) on the aromatic nucleus is(are) preferably free also in the N-acyl derivatives because of the same reason as above.

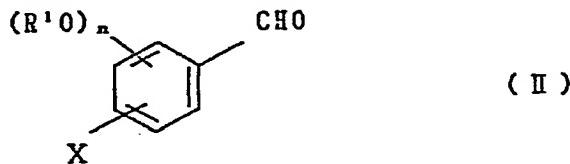
The halogen atom includes anyone of fluorine, chlorine, bromine and iodine atoms, and in particular, the bromine atom-substituted compounds are especially preferred.

Typical examples of the compounds of the formula (I), which are preferred in the present invention, are given below.

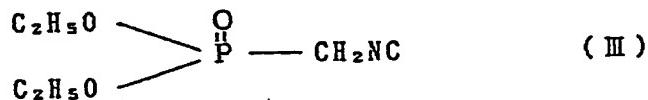
(2-isobutynyl)-2,5-dihydroxybenzene,
(2-isobutynyl)-2,3-dihydroxybenzene,
(2-isobutynyl)-2,4-dihydroxybenzene,
(2-isobutynyl)-2,6-dihydroxybenzene,
(2-isobutynyl)-3,4-dihydroxybenzene,
(2-isobutynyl)-3,5-dihydroxybenzene,
(2-isobutynyl)-2,5-dimethoxybenzene,
(2-isobutynyl)-2,5-diacetoxybenzene,
(2-isobutynyl)-2-hydroxy-3-methoxybenzene,
(2-isobutynyl)-2-dihydroxybenzene,
(2-isobutynyl)-2-hydroxy-3-bromobenzene,
(2-isobutynyl)-2,3,4-trihydroxybenzene,

(2-isonitrilevinyl)-2,4,5-trihydroxybenzene,
 (2-isonitrilevinyl)-2,3,5-trihydroxybenzene,
 (2-isonitrilevinyl)-2,3,6-trihydroxybenzene,
 (2-isonitrilevinyl)-3,4,5-trihydroxybenzene,
 and further, 2-formamidovinyl, 2-formamidoethylene or 2-alkanoylaminovinyl derivatives corresponding to the said compounds, with the exception of (2-formamidovinyl)-2,5-dihydroxybenzene and (2-formamidoethyl)-2,5-dihydroxybenzene.

Next, the compounds of the present invention can be obtained by reacting a compound of a formula (II):



(in which R^1 , n and X have the same meanings as mentioned above) and a diethyl isocyanomethyl-phosphite of a formula (III):



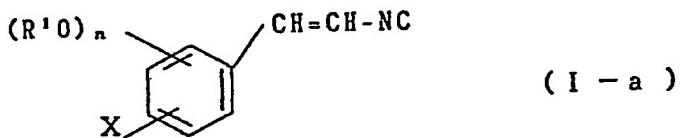
in the presence of a base, by a per se known Wittig reaction, optionally followed by acid hydrolysis and further hydrogenation of the resulting product.

The said Wittig reaction may be carried out for several hours at about -78°C to 0°C in the presence of a substantially inactive polar solvent such as N,N -dimethylformamide, diethylether, dioxane or tetrahydrofuran (hereinafter referred to as "THF").

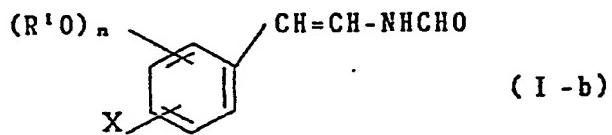
0238868

The reaction is preferably carried out in a water-free system; and as the base substance can be used a metal base such as sodium bis(trimethylsilyl)amide, sodium hydride, butyl lithium, potassium t-butoxide, etc.

By the said reaction are obtained the compounds of a formula (I-a), which fall within the scope of the compounds of the present invention:



(in which R¹, n and X have the same meanings as given above). The compounds of the said formula (I-a) are, after isolated or not isolated from the reaction mixture in a conventional manner, subjected to acid-hydrolysis to give the compounds of a formula (I-b), which also fall within the scope of the compounds of the present invention.



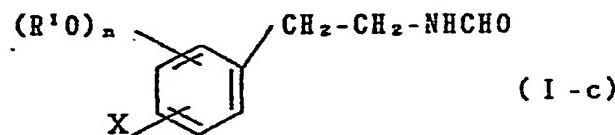
(in which R¹, n and X have the same meanings as given above).

The said hydrolysis reaction can be carried out in the co-presence of an organic solvent, such as ethyl acetate, diethylether, benzene, methyl alcohol, ethyl

0238868

alcohol, THF, dioxane or chloroform, and water, using a catalyst of an organic acid, such as trifluoroacetic acid, acetic acid, oxalic acid or malonic acid, or an inorganic acid, such as hydrochloric acid, sulfuric acid or phosphoric acid, under the condition of about -10°C to 40°C. The reaction time is not critical but varies, depending upon the solvent and acid catalyst used and the reaction temperature. In general, the reaction may be completed in several hours to several days.

Further, the compounds of the formula (I-b) can be hydrogenated in the presence of a catalyst to give the other compounds of a formula (I-c), which also fall within the scope of the present invention:



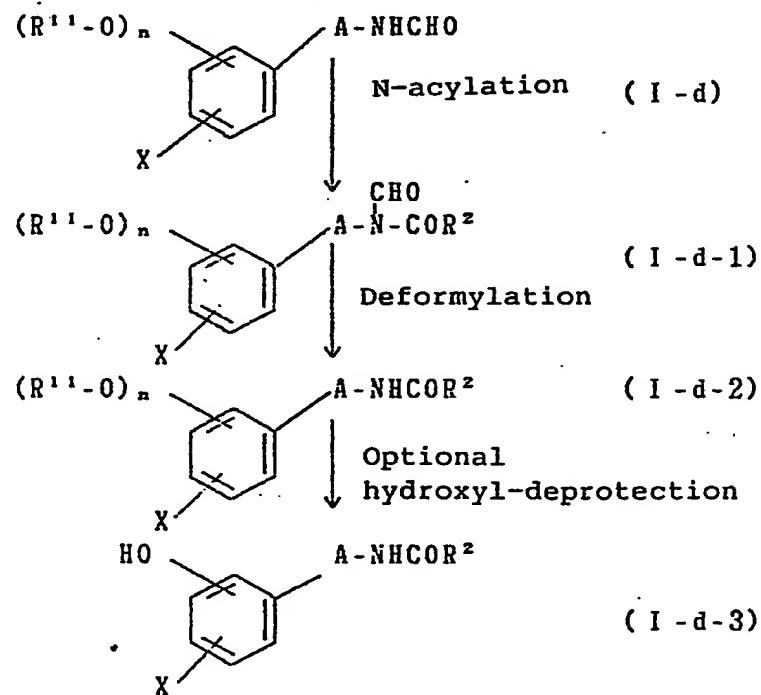
(in which R¹, n and X have the same meanings as given above).

The hydrogenation can be carried out by a per se known reaction which is generally used for the hydrogenation of an ethylene chain. For instance, the compound of the formula (I-b) is reacted with a hydrogen in the presence of a catalyst such as platinum oxide or palladium-carbon, using a solvent of a lower alkanol, such as methyl alcohol, ethyl alcohol or isopropyl alcohol, or water.

The compounds of the present invention as obtained in each of the above-mentioned steps can be isolated by a per se known isolation and purification method. For instance, the isolation can rationally be carried out by chromatography with silicagel.

In addition, the compounds of the formula (I-b) or (I-c) where R¹ is a lower alkanoyl group or a lower alkyl group can be converted into the corresponding N-acyl derivatives, which fall the scope of the compounds of the present invention, in accordance with the following reaction procedure.

Reaction process:



0238868

In the said reaction formulae, R¹¹ represents a lower alkanoyl group or a lower alkyl group; A represents a formula -CH=CH- or -CH₂-CH₂-; and R², X and n have the same meanings as given above.

The reaction in each reaction step of the above-mentioned reaction procedure is carried out in accordance with a per se known method, which is concretely as follows:

(i) N-acylation:

This reaction is completed by reacting the compound of the formula (I-d) and an acylating agent such as a lower fatty acid, an acid anhydride or an acid halide. The reaction can be carried out by means of a per se known acylation method, for example, in the presence of a basic substance or the like.

(ii) Deformylation:

The reaction in this step is one to remove the N-formyl group from the compound of the formula (I-d-1), which can be carried out by means of a per se known deformylation method. For instance, the compound is treated with an inorganic acid such as hydrochloric acid or sulfuric acid in a solvent of a lower alcohol such as methanol or ethanol, at room temperature for several hours.

(iii) Hydroxyl-deprotection:

The reaction in this step is to selectively remove the lower alkanoyl group of the phenolic hydroxyl group in the compound of the formula (I-d-2), which can be

0238868

carried out in the same manner as the above-mentioned step (ii) with the exception that the concentration of the acid used is elevated.

In each of the above-mentioned reaction steps, the isolation and purification of the product from the reaction mixture can be carried out by means of a per se known chromatography, solvent extraction of the like method.

The compounds of the present invention thus obtained have significant physiological activities, as mentioned hereinafter, and thus, these are useful as biochemical reagents, antimicrobial substances or antitumor substances.

Specifically, the effects of the compounds of the present invention are illustrated below.

(A) TPK-I activity:

The tyrosine-specific protein-kinase activity was assayed by means of a modified method of ELISA by G. Carpenter, et al. [(The Journal of Biological Chemistry, 254, 4874-4891 (1979)], where a membrane fraction of human epidermal carcinoma cells A-431 was used as the tyrosine-specific protein-kinase activator of an epidermal growth factor receptor. Specifically, 1mM MnCl₂, 100ng epidermal growth factor, 40μg A-431 membrane fraction, 7.5μg albumin, 3μg histone and 50μl of 20mM Hepes buffer (pH 7.4) containing MH435-A or MH435-B were pre-incubated at 0°C for 10 minutes, and 10μl of (γ -³²P)-adenosine triphosphate (0.25mCi/0.125ml)

0238868

was added thereto and reacted at 0°C for 30 minutes. 50μl of the reaction mixture was sampled and absorbed to Wattman No. 3MM filter membrane and dipped in an ice-cooled TCA. After kept for 30 minutes, the filter membrane was taken out and washed out TCA, ethanol and ether, and the count of ^{32}P as stuck to the said filter membrane was measure (a). The same reaction and treatment were carried out, where the test substance only was excluded, and the count of ^{32}P on the filter was measured in the same manner (b). Further, the values as measured in the cases where the membrane fraction was excluded were (a') and (b'), respectively. Thus, the percentage of the inhibition of the epiderman growth factor receptor-kinase was calculated from the formula of $[(a-a')/(b-b')] \times 100$.

The TPK-I activity of typical compounds of the present invention was extremely high at a low concentration, as shown in the following Table.

0238868

Table - 1

Compound No.	TPK-I IC ₅₀ (μ g/ml)
1	6.25
2	>6.4
3	>10
9	>6.4
10	>30
11	>6.4
12	>100
13	0.3
14	1.3
18	3.0

(B) Growth inhibition activity against cultured carcinoma cells:

Each of L 1210 leukemia cells, IMC carcinoma cells, Ki-NRK, tsSre-NRK and A-431 cells were put on a dish ($3-5 \times 10^4$ cells/dish) and incubated for one day (37°C , CO_2 concentration: 5%), and then, MH435-A was added thereto and further incubated for 3 days under the same condition. After the incubation, the number of the cells was counted and the activity was evaluated.

The 50%-inhibition concentration (IC₅₀) of typical compounds of the present invention against the growth of the said cells is given in the following Table, which indicates the usable effect of the compounds as a antitumor substance.

0238868

Table -2

Compound No.	L 1210 IC ₅₀ (μ g/ml)
1	1.4
2	0.25
3	0.65
9	12
10	62
11	23
12	30
13	2.2
14	22
18	3.0

(c) Antimicrobial activity:

The minimum growth inhibition concentration (MIC) of the compounds of the present invention against various bacteria was measured by means of an agar dilution method using Muller-Hinton medium, which is shown in the following Table.

Table -3

Compound No.	Tested Bacteria	
	Staphylococcus aureus Smith MIC (μ g/ml)	Psudomonas aeruginosa A3 MIC (μ g/ml)
1	25	50
2	6.25	12.5
3	3.12	25
9	100	>50
10	>100	>100
11	>100	>50
12	>100	>50
13	100	100
14	>50	25

As shown in the above Tables, the compounds of the present invention have high TPK-I activity, growth inhibition activity against cultured carcinoma cells and antimicrobial activity at a low concentration, and therefore, these are useful not only as biochemical reagents but also as antitumor agents and antimicrobial agents.

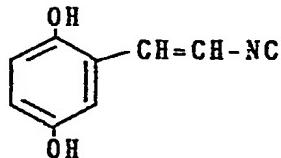
The present invention will be explained in greater detail by reference to the following examples, which, however, are not intended to be interpreted as limiting the scope of the present invention.

0238868

Example 1:

Preparation of 2-(2,5-dihydroxyphenyl)vinylisocyanide

(1):



A solution of anhydrous tetrahydrofuran (THF) (2ml) containing 132.8mg of dimethyl isocyanomethylphosphite was dropwise added to a solution of anhydrous THF (2ml) containing 137.3mg of sodium bis(trimethylsilyl)amide, while stirred at -78°C, under the substitution by nitrogen gas. The whole was stirred for 20 minutes at the same temperature, and then, a solution of anhydrous THF (1ml) containing 20.7mg of 2,5-dihydroxy-benzaldehyde was added thereto and reacted for 3 hours. Next, 100 μ l of acetic acid was added, and the reaction mixture was diluted with 20ml of ethyl acetate and washed with 0.1M phosphate buffer (pH 7.0) for liquid-separation. The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure until the amount of the solvent became 3ml, and thereafter, this was purified by preparative T.L.C. (toluene-acetone, 2:1) to obtain 14.0mg of the said product (Yield: 59%).

R_f = 0.17 (toluene-acetone, 2:1)

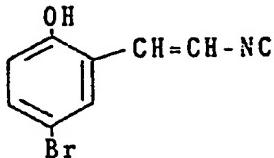
IR_vKBr_{max} cm⁻¹ : 3280, 2140, 1590, 1505

0238868

Example 2:

Preparation of

2-(5-bromo-2-hydroxyphenyl)vinylisocyanide (2):



Using 342mg of sodium bis(trimethylsilyl)amide, 331mg of diethyl isocyano-phosphite and 149mg of 5-bromo-2-hydroxybenzaldehyde, these were reacted and treated in the same manner as the Example 1. The crude product was purified by preparative T.L.C. (toluene-acetone, 3:2), to obtain 106.8mg of the said product (Yield: 65.0%).

R_f = 0.69 (toluene-acetone, 3:2)

IR_vKBr_{max} cm⁻¹ : 3190, 2140, 1595, 1495

NMR_δppm(CDCl₃):

5.52(1H,s)

6.55(1H,d, J=15Hz)

6.70(1H,d, J=9Hz)

7.02(1H,d, J=15Hz)

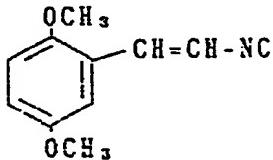
7.35(1H,d, J=9Hz)

7.40(1H,s)

0238868

Example 3:

Preparation of 2-(2,5-dimethoxyphenyl)vinylisocyanide
(3):



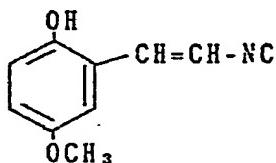
137.3mg of sodium bis(trimethylsilyl)amide, 132.8mg of diethyl isocyanomethyl-phosphite and 62mg of 2,5-dimethoxybenzaldehyde were used, and these were reacted and treated in the same manner as the Example 1. The crude product was purified by preparative T.L.C. (toluene-ethyl acetate, 8:1), to obtain 44.5mg of the said product (Yield: 63.0%).

R_f = 0.73 (toluene-ethyl acetate, 8:1)

Example 4:

Preparation of

2-(2-hydroxy-5-methoxyphenyl)vinylisocyanide (4):



137.3mg of sodium bis(trimethylsilyl)amide, 132.8mg of diethyl isocyanomethyl-phosphite and 50mg of 2-hydroxy-5-methoxybenzaldehyde were used, and these were reacted and treated in the same manner as the Example 1. After purified by preparative T.L.C.

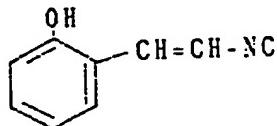
0238868

(toluene-acetone, 1:1), 38.2mg of the said product was obtained (Yield: 67.0%).

RF = 0.79 (toluene-acetone, 1:1)

Example 5:

Preparation of 2-(2-hydroxyphenyl)vinylisocyanide (5):

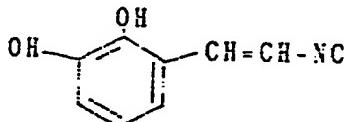


114mg of sodium bis(trimethylsilyl)amide, 111mg of diethyl isocyanomethyl-phosphite and 40 μ l of 2-hydroxybenzaldehyde were used, and these were reacted and treated in the same manner as the Example 1. After purified by preparative T.L.C. (toluene-ethyl acetate, 8:1), 37.7mg of the said product was obtained (Yield: 68.5%).

RF = 0.44 (toluene-ethyl acetate, 8:1)

Example 6:

Preparation of 2-(2,3-dihydroxyphenyl)vinylisocyanide (6):



342mg of sodium bis(trimethylsilyl)amide, 331mg of diethyl isocyanophosphite and 64.2mg of 2,3-dihydroxybenzaldehyde were used, and these were reacted and treated in the same manner as the Example 1. After

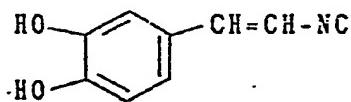
0238868

purified by preparative T.L.C. (chloroform-methanol, 10:1), 46.8mg of the said product was obtained (Yield: 62.5%).

R_f = 0.33 (chloroform-methanol, 10:1)

Example 7:

Preparation of 2-(3,4-dihydroxyphenyl)vinylisocyanide (7):

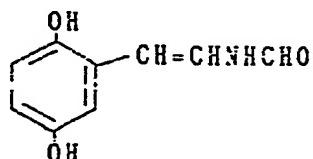


In the same manner as the Example 6 with the exception that 64.2mg of 3,4-dihydroxybenzaldehyde was used, the materials were reacted and purified to obtain 49.0mg of the said product (Yield: 65.5%).

R_f = 0.46 (chloroform-methanol, 10:1)

Example 8:

Preparation of (2-formamidovinyl)2,5-dihydroxybenzene (8):



5ml of 0.1N hydrochloric acid-aqueous solution was added to 5ml of ethyl acetate solution containing 10.0mg of 2-(2,5-dihydroxyphenyl)vinylisocyanide and vigorously stirred for 2 days. The organic layer was washed with a saturated sodium chloride-aqueous solution and then,

0238868

dried with anhydrous sodium sulfate, and the solvent was distilled out under reduced pressure. Next, the resulting residue was purified by preparative T.L.C. (toluene-acetone, 1:1), to obtain 4.7mg of the said product (Yield: 42.4%).

R_f = 0.37 (toluene-acetone, 1:1)

m.p.: 78-82°C

IR_vKBr_{max} cm⁻¹ : 1650, 1540, 1510, 1400,
1320, 1260, 1200

NMR δppm(acetone-d₆):

6.64(1H,d, J=15.0Hz)

6.51(1H,dd, J=3.0Hz, 9.0Hz)

6.68(1H,d, J=9.0Hz)

6.80(1H,d, J=3.0Hz)

7.63(1H,dd, J=15.0Hz, J=11.0Hz)

7.72(1H,s)

8.02(1H,s)

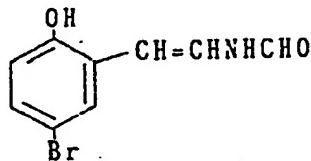
8.17(1H,s)

9.30(1H,br)

Example 9:

Preparation of

(2-formamidovinyl)-5-bromo-2-hydroxybenzene (9):



Using 20mg of 2-(5-bromo-2-hydroxyphenyl)vinyl-isocyanide, the materials were reacted and treated in

0238868

the same manner as the Example 8. After purified by preparative T.L.C. (toluene-acetone, 3:2), 9.7mg of the said product was obtained (Yield: 45%).

R_f = 0.37 (toluene-acetone, 3:2)

m.p. 146.5-148.0°C

IR_VKBr_{max} cm⁻¹ : 3370, 3200, 1685, 1675,
1640, 1515, 1485, 1405

NMR_δppm(acetone-d₆):

6.46(1H,d, J=15.0Hz)

6.85(1H,d, J=9.0Hz)

7.17(1H,dd, J=2.0Hz, J=9.0Hz)

7.48(1H,d, J=2.0Hz)

7.90(1H,dd, J=9.0Hz, J=15.0Hz)

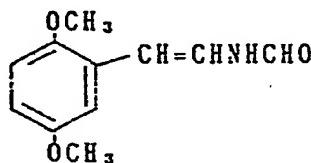
8.14(1H,s)

8.99(1H,s)

9.35(1H,br)

Example 10

Preparation of (2-formamidovinyl)-2,5-dimethoxybenzene (10):



Using 20.0 mg of 2-(2,5-dimethoxyphenyl)vinylisocyanide, the materials were reacted and treated in the same manner as the Example 8. After purified by preparative T.L.C. (toluene-acetone,

0238868

1:1), 10.1mg of the said product was obtained (Yield: 46.5%).

R_f = 0.58 (toluene-acetone, 1:1)

m.p.: 83.5-85.0°C

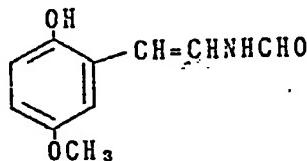
IR_{VKBr} max cm⁻¹ : 3300, 1710, 1685, 1665,
1530, 1510, 1230

NMR_δ ppm (CDCl₃):

3.78(s), 3.81(s), 3.82(s), 6.23(d, J=15.0Hz),
6.43(d, J=15.0Hz), 6.79(s), 6.93(d, J=3.0Hz),
8.22(s)

Example 11:

Preparation of (2-formamidovinyl)-2-hydroxy-5-methoxybenzene (11):



Using 30mg of 2-(2-hydroxy-5-methoxyphenyl)vinyl-isocyanide, the materials were reacted and treated in the same manner as the Example 8. After purified by preparative T.L.C. (toluene-acetone, 1:1), 13.7mg of the said product was obtained (Yield: 41.3%).

R_f = 0.50 (toluene-acetone, 1:1)

m.p.: 127.5-129.0°C

IR_{VKBr} max cm⁻¹ : 3290, 3200, 1695, 1655,
1520, 1445, 1225

NMR_δ ppm (CDCl₃+acetone-d₆):

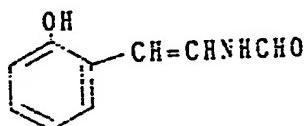
3.76(3H,s)

0238868

6.50(1H,d, J=15.0Hz)
6.61(1H,dd, J=3.0Hz, J=9.0Hz)
6.82(1H,d, J=9.0Hz)
6.88(1H,d, J=3.0Hz)
7.70(1H,s)
7.72(1H,d, J=15.0Hz)
8.22(1H,s)
8.92(1H,br)

Example 12:

Preparation of (2-formamidovinyl)-2-hydroxybenzene (12):



25mg of 2-(2-hydroxyphenyl)vinylisocyanide was used, and the materials were reacted and treated in the same manner as the Example 8. After purified by preparative T.L.C. (toluene-ethyl acetate, 3:1), 13.6mg of the said product was obtained (Yield: 48.5%).

R_f = 0.12 (toluene-ethyl acetate, 3:1)

m.p.: 118.0-119.0°C

IR_VKBr_{max} cm⁻¹ : 3350, 3200, 1660, 1645, 1640,
1530, 1455, 1385, 1255

NMR_δppm(CDCl₃+acetone-d₆):

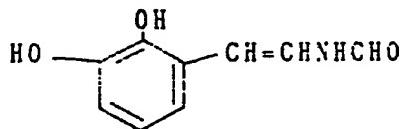
6.50(1H,d, J=15.0Hz)
6.82(1H,dt, J=2.0Hz, J=8.0Hz)
6.88(1H,dd, J=2.0Hz, J=8.0Hz)
6.98(1H,dt, J=2.0Hz, J=8.0Hz)

0238868

7.32(1H,dd, J=2.0Hz, J=8.0Hz)
7.72(1H,dd, J=15.0Hz, J=11.0Hz)
8.01(1H,br)
8.21(1H,s)
8.60(1H,br)

Example 13:

Preparation of (2-formamidovinyl)-2,3-dihydroxybenzene
(13):



25mg of 2-(2,3-dihydroxyphenyl)vinylisocyanide was used, and the materials were reacted and treated in the same manner as the Example 8. After purified by preparative T.L.C. (chloroform-methanol, 10:1), 12.2mg of the said product was obtained (Yield: 44.2%).

RF = 0.12 (chloroform-methanol, 10:1)

UVλ_{MeOH}_{max} nm (δ): 282(18600)

225(14500)

207(12000)

IRν_{KBr}_{max} cm⁻¹ : 3350, 1670, 1655, 1485, 1390,
1290

NMRδ ppm(acetone-d₆):

6.51(1H,d, J=15.0Hz)
6.6-7.0(3H,m)
7.70(1H,dd, J=15.0Hz, J=10.5Hz)
8.22(1H,s)

0238868

7.60(1H,br)

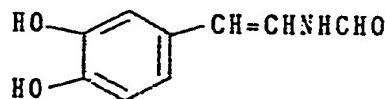
8.50(1H,br)

9.25(1H,br)

Example 14:

Preparation of (2-formamidovinyl)-3,4-dihydroxybenzene

(14):



25mg of 2-(3,4-dihydroxyphenyl)vinylisocyanide was used, and the materials were reacted and treated in the same manner as the Example 8. After purified by preparative T.L.C. (chloroform-methanol, 10:1), 15.8mg of the said product was obtained (Yield: 46.3%).

R_f = 0.29 (chloroform-methanol, 10:1)

m.p.: 183-187°C

IR_{VKBr} max cm⁻¹ : 3340, 3150, 1670, 1640, 1600,
1500, 1390, 1280, 1200

NMR_δ ppm(acetone-d₆):

6.20(1H,d, J=15.0Hz)

6.7-6.9(3H,m)

7.35(1H,d, J=15.0Hz)

8.19(1H,s)

0238868

Example 15:

Preparation of (2-formidoethyl)-2,3-dihydroxybenzene

(15):

5mg of platinum oxide was added to methanol solution (2ml) containing 4.5mg of (2-formamidovinyl)-2,3-dihydroxybenzene and stirred for 2 hours in hydrogen atmosphere. After the catalyst was filtrated out, the reaction solution was concentrated under reduced pressure, and the residue was purified by preparative T.L.C. (toluene-acetone, 1:1) to obtain 4.2mg of the said product (Yield: 92.3%).

R_f = 0.35 (toluene-acetone, 1:1)

NMR_δppm(acetone-d₆-methanol-d₄):

2.82(2H,t)

3.48(2H,t)

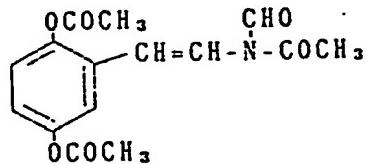
6.5-6.9(3H,m)

8.15(1H,s)

Example 16:

Preparation of (3-aza-3-formyl-4-oxopentenyl)-2,5-

diazetoxy benzene (16):



10ml of acetic anhydride, 30ml of triethylenamin and 5mg of 4-dimethylaminopyridine were added to 2ml of anhydrous methylene chloride-solution containing 14mg of (2-formamidovinyl)-2,5-diacetoxybenzene and stirred for

0238868

4 hours at room temperature, and then, the reaction mixture was put into 20ml of ethyl acetate and subjected to liquid-separation with 0.01N-hydrochloric acid and saturated sodium chloride-aqueous solution, and then, washed. The organic layer was dried with anhydrous sodium sulfate, and the solvent was distilled out under reduced pressure. The resulting residue was purified by preparative T.L.C. (toluene-acetone, 2:1) to obtain 15.0mg of the said product (Yield: 92%).

R_f = 0.82(chloroform-methanol, 10:1)

NMRδppm(CDCl₃):

2.28(3H,s)

2.32(3H,s)

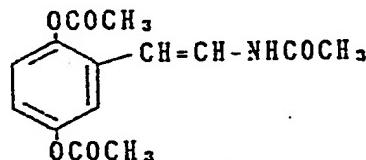
2.43(3H,s)

6.9-7.3(5H,m)

9.30(1H,s)

Example 17:

Preparation of (2-acetamidovinyl)-2,5-diacetoxybenzene (17):



20μl of 0.1N-hydrochloric acid was added to 2ml of methanol solution containing 15mg of the compound (16) and stirred for 1 hour at room temperature. Next, 20ml of ethyl acetate was added thereto and the resulting

0238868

mixture was subjected to liquid-separation with saturated sodium chloride-aqueous solution and washed.

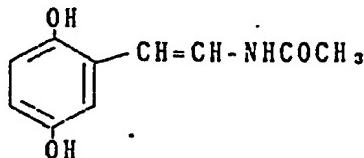
The organic layer was dried with anhydrous sodium sulfate, and then, the solvent was distilled out, and the residue obtained was purified by preparative T.L.C. (chloroform-methanol, 10:1) to give 12.5mg of the said product (Yield: 91.7%).

R_f = 0.53 (chloroform-methanol, 10:1)

Example 18:

Preparation of (2-acetamidovinyl)-2,5-dihydroxybenzene

(18):



50μl of 0.1N-hydrochloric acid was added to 2ml of methanol solution containing 12.5mg of the compound (17) and stirred for 4 hours at room temperature, and then, 30ml of ethyl acetate was added thereto. The resulting mixture was subjected to liquid-separation with saturated sodium chloride-aqueous solution and washed.

The organic layer was dried with anhydrous sodium sulfate and then, the solvent was distilled out. The resulting residue was purified by preparative T.L.C. (chloroform-methanol, 10:1), to obtain 7.3mg of the said product (Yield: 84%).

R_f = 0.1 (chloroform-methanol, 10:1)

MNR_δppm(acetone-d₆):

0238868

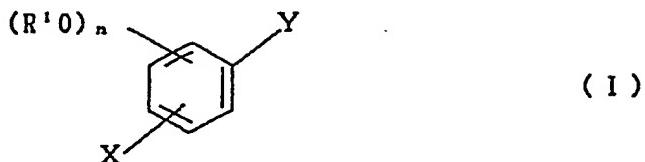
1.99(3H,s)
6.35(1H,d, J=15Hz)
6.50(1H,dd, J=9Hz, J=3Hz)
6.70(1H,d, J=9Hz)
6.82(1H,d, J=3Hz)
7.60(1H,dd, J=15Hz, J=10.5Hz)
9.2(1H,br)

As apparent from the above description, the compounds of the present invention can inhibit the tyrosine-specific protein-kinase activity, and these are new substances which have excellent antimicrobial activity and antitumor activity. The present invention provides the new substances having the said valuable property.

0238868

WHAT IS CLAIMED IS:

1. Compounds of a general formula (I):



(in which R¹ represents a hydrogen atom, a lower alkanoyl group or a lower alkyl group; n represents a positive integer of 1 to 3;

X represents a hydrogen atom or a halogen atom;

Y represents a group of formula -CH=CH-NC, -CH=CH-NHR² or -CH₂-CH₂-NHR² (where R² represents a formyl group or a lower alkanoyl group.);

with the exception of the cases where X is hydrogen atom, (R¹O)_n group is 2,5-dihydroxy group and Y is -CH=CH-NHCHO or -CH₂-CH₂-NHCHO).

2. Compounds as claimed in claim 1, wherein Y in the formula (I) is -CH=CH-NC.

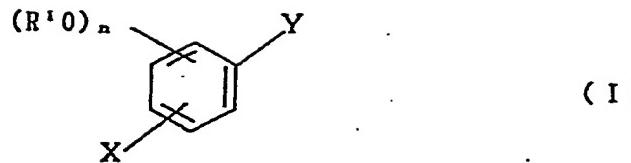
3. Compounds as claimed in claim 1, wherein Y in the formula (I) is -CH=CH-NHCHO.

4. Compounds as claimed in claim 1, wherein Y in the formula (I) is -CH=CH-NHCOCH₃.

5. Compounds as claimed in anyone of claims 1 to 4, wherein n in the formula (I) is a positive integer of 2.

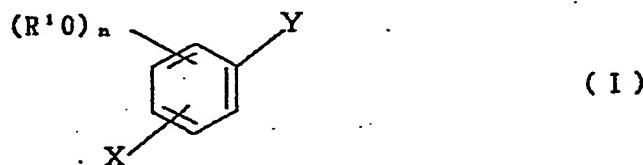
6. A tyrosine-specific protein-kinase inhibitor containing a compound of a general formula (I):

0238868



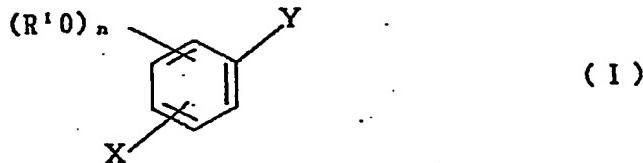
(in which R¹, n, X and Y have the same meanings as given above).

7. A tumoricide containing a compound of a general formula (I):



(in which R¹, n, X and Y have the same meanings as given above).

8. A bactericide containing a compound of a general formula (I):



(in which R¹, n, X and Y have the same meanings as given above).

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.